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Part II

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, HHS.

ACTION: Notice of actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH, under the 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules (45 FR 6724).

EFFECTIVE DATE: July 29, 1980.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205. (301) 496–6051.

SUPPLEMENTARY INFORMATION: I am promulgating today several major actions under the NIH Guidelines for Research Involving Recombinant NDA Molecules. These proposed actions were published for comment in the **Federal Register** of April 30, 1980, and reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on June 5–6, 1980. In accordance with Section IV–E–1–b of the NIH Guidelines, I find that these actions comply with the Guidelines and present no significant risk to health or the environment. I am deferring action on one major action, recommended for approval by the RAC, proposing the field testing of corn plants (*Zea mays*) into which corn recombinant DNA has been added using an *E. coli* or *S. cerevisiae* vector. A final decision on this proposal will be reached after the receipt of additional information.

Part I of this announcement provides background information on the actions.

Part II provides a summary of the major actions.

I. Decisions on Actions Under Guidelines

I–A. Proposal To Include Saccharomyces Cerevisiae Hose-Vector Systems Under Section III–O of the Guidelines

Dr. Jane Setlow of the Brookhaven National Laboratory, at the March 6–7, 1980 Recombinant DNA Advisory Committee (RAC) meeting, proposed that NIH consider classifying experiments involving laboratory strains of *Saccharomyces cerevisiae* as host-vector systems under Section III–O of the Guidelines. Dr. Setlow advanced the following arguments to support this proposal: (1) *S. cerevisiae* is nonpathogenic; (2) *S. cerevisiae* does not implant in the mammalian intestine; (3) the dilute conditions in which *S. cerevisiae* is found in nature are extremely unfavorable for mating; and (4) laboratory strains do not efficiently compete with wild type strains *S. cerevisiae*. She added that *S. cerevisiae* does not have a biological association with man.

The RAC discussed this issue at the March 6–7, 1980 meeting, and agreed that the proposal should be published in the **Federal Register** to permit thirty days of comment. Accordingly, proposed language which would amend two sections of the Guidelines, appeared in the **Federal Register** of April 30, 1980 [45 FR 28905]. This involved a proposed substitution for Section III–O of the Guidelines and a deletion in Appendix D, as follows:

III–O. *Classification of Experiments Using E. coli K–12 and Saccharomyces cerevisiae Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K–12 host-vector systems; others employ the *S. cerevisiae* host-vector systems. These are the systems for which we have the most experience and knowledge.

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are prohibited (see Section I–D).

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are exempt from the Guidelines (see Section I–E).

Other experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, an HV1 host-vector system [i.e., for experiments using *E. coli* K–12 (a) the *E. coli* host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid bacteriophages or non-conjugative plasmids [49] shall be used as vectors. For experiments in *S. cerevisiae*, laboratory strains shall be used.] for these experiments no Memorandum of Understanding and Agreement (MUA) as described in Section IV–D–1–c need be submitted, nor is any registration with NIH necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, and (c) the hosts and vectors to be used. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals but such review is not required prior to initiation of experiments. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 or *S. cerevisiae* efficiently express as a protein product the information carried in any gene derived from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.

Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).

For those not on the Appendix A list but which exchange genetic information (35) with *E. coli*, experiments may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a nonconjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or integrated, or generalized transducing phages.

The language "*HV1-Unmodified laboratory strains of *Saccharomyces cerevisiae*" would be deleted from Appendix D.

During the thirty-day comment period, no comments were received.

At the June 5–6, 1980 meeting, the RAC once again discussed this issue. It was noted that *S. cerevisiae* does not colonize animals, large amounts of yeast are regularly consumed by humans with no ill effects, *S. cerevisiae* does not appear to express mammalian genes, and the probability that genetic material will be transferred from or otherwise disseminated by laboratory strains of *S. cerevisiae* is very low.

The RAC suggested that the language of the sentence of the proposed amended Section III–O which begins, "an exception, however * * *" might lead to confusion over the status of cloning *S. cerevisiae* DNA in *S. cerevisiae*. A motion that the phrase "or *S. cerevisiae*" be deleted from this sentence passed by a vote of fifteen in favor, one opposed, and three abstentions. The amended sentence would read:

* * *An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have *E. coli* K–12 efficiently express as a protein product the information carried in any gene derived from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.

The proposal to include *S. cerevisiae* host-vector systems under Section III–O of the Guidelines as published in the **Federal Register** of April 30, 1980 with the amendment noted above, was accepted by the RAC by a vote of fourteen in favor, two opposed, and two abstentions.

I accept this recommendation.

In addition to the deletion of reference to HV1 *S. cerevisiae* in Appendix D of the Guidelines, reference to HV2 *S. cerevisiae* has also been deleted from Appendix D. Added to Appendix D,

however, is reference to RUB 331 which has been certified as the host component of *B. subtilis* HV1 systems. Added to Appendix E is a description of certified EK2 and HV2 *E. coli* and *S. cerevisiae* systems.

I–B. Proposal To Amend Section III–O of the Guidelines To Permit Use of Ff Phages

In January 1980 (45 FR 6724) the National Institutes of Health (NIH) promulgated revised Guidelines. The revised Guidelines included a Section, III–O, "Classification of Experiments Using the *E. coli* K–12 Host-Vector Systems," which reads, in part, as follows:

* * * experiments using *E. coli* K–12 shall use P1 physical containment and * * * an EK1 host-vector system [i.e., (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages and (b) lambda or lambdoid bacteriophages or nonconjugative plasmids shall be used as vectors].* * *

No reference to the use of Ff bacteriophages (filamentous single-strand male-specific bacteriophages such as M13 and fd) was made in Section III–O. Consequently, when the revised Guidelines were promulgated in January 1980, investigators were informed that Ff bacteriophages might continue to be used as a component of EK1 systems at the containment levels specified by the 1978 Guidelines.

In December 1979, the RAC was asked to consider how *E. coli* K–12 host-vector systems employing Ff phages should be treated under the 1980 Guidelines. In response to this NIH request, the Host-Phage Subcommittee drafted a recommendation which was presented to the full RAC at the March 6–7, 1980 meeting. The RAC agreed that the proposed amended language of Section III–O should be published in the **Federal Register** for thirty days of comment. Accordingly, the following language appeared in the **Federal Register** of April 30, 1980 [45 FR 28904]:

* * * experiments using *E. coli* K–12 shall use P1 physical containment and, * * * an EK1 host-vector system [i.e., (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages and (b) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids shall be used as vectors].* * *

No comments on this proposal were received during the thirty-day comment period.

At the June 5–6, 1980 meeting, the RAC once again considered this issue. The safety of Ff phages was discussed. By a vote of fourteen in favor, none opposed, the RAC recommended that the Host-Phage Subcommittee proposal to include Ff phages under Section III–O be accepted.

I accept this recommendation.

I–C. Proposed Changes To Allow Recombinant DNA Experiments With Class 3 Organisms and Certain Plant Pathogens

In December 1979, the National Institute of Allergy and Infectious Diseases (NIAID) requested that the NIH consider whether, and under what conditions, recombinant DNA technology might be used to study CDC Class 3 etiological agents. Currently Section I–D–1 of the Guidelines prohibits:

Formation of recombinant DNAs derived from the pathogenic organisms classified [1] as Class 3, 4 or 5 [2] or the cells known [2A] to be infected with such agents, regardless of the host-vector system used.

This issue was presented to the RAC at its December 6–7, 1979 meeting and a working group was appointed to study the issue. A draft proposal which would revise the Guidelines so as to remove Class 3 agents from Section I–D–1 of the Guidelines was subsequently presented to the full RAC at its March 6–7, 1980 meeting.

At the March 6–7, 1980 meeting, a request from Dr. Clarence Kado of the University of California, Davis, to delete Section I–D–3 of the Guidelines was also presented to the RAC. Section I–D–3 prohibits:

Deliberate creation by the use of recombinant DNA of a plant pathogen with increased virulence and host range beyond that which occurs by natural genetic exchange. [2A]

During the discussion of this issue it was suggested that the mechanism used to remove CDC Class 3 agents from Section I–D–1 of the Guidelines might be appropriate for removing recombinant DNA experiments involving certain plant pathogens from Section I–D–3. Accordingly, the proposals have been combined.

The proposed changes would remove recombinant DNA experiments with CDC Class 3 organisms and certain recombinant DNA experiments with plant pathogens from the prohibited classes of experiments. The proposal would permit experiments with CDC Class 3 organisms to be conducted at P3 containment in *E. coli* K–12 EK1 host-vector systems. The containment levels for other recombinant DNA experiments would be assigned by the Director, NIH after review by the RAC.

It is felt that the proposal would reduce administrative procedures, thereby increasing flexibility and encouraging the use of recombinant techniques to elucidate the pathogenic process. It was reasoned that P3 containment, which is considered adequate for work with the pathogenic organisms themselves, should be adequate, or more than adequate, for work with *E. coli* K–12 containing recombinant DNA from these organisms.

The proposed amendments to the Guidelines appeared in the **Federal Register** of April 30, 1980 [45 FR 28904] as follows:

A. Section I–D–1 is to be amended to read:

I–D–1. Formation of recombinant DNAs derived from pathogenic organisms classified [1] as Class 4 or 5 or from cells known [2A] to be infected with such agents, regardless of the host-vector system used.

B. Section I–D–3 is to be deleted.

C. Section I–E is to be amended to read:

I–E. Exemptions. It must be emphasized that the following exemptions [4] are not meant to apply to experiments described in Sections I–D–1 to I–D–5 as being prohibited.

In addition, any recombinant DNA molecules involving DNA from Class 3 organisms (1) or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange, are not exempt unless specifically so designated by NIH under Section I–E–5.

D. Section III. After the last sentence, "The use of higher levels of biological containment * * * for the purposes of the experiment," the following new paragraph is to be added:

Experiments involving recombinant DNA from Class 3 organisms (1) or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K–12 EK1 hosts (see Section III–O). Containment levels for all other experiments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen will be determined by NIH (See Section IV–E–1–b–2–e).

E. Section III–O. After the third paragraph, 'Some experiments * * * are exempt from the Guidelines (see Section I–E),' the following new paragraph is to be added:

Experiments using *E. coli* K–12 EK1 host-vector systems and DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment or at a lower level as specified by NIH (See section IV–3–1–b–2–e).

F. A new Section IV–E–1–b–2–e is to be added as follows:

IV–E–1–b–2–e. Assigning containment levels for experiments with recombinant DNA from Class 3 organisms [1] and for experiments which increase the host-range and virulence of plant pathogens.

G. Section V, footnote 2, is to be amended to read:

2. For experiments using Vesicular Stomatitis virus (VSV), contact the NIH Office of Recombinant DNA Activities.

H. Section V, footnote 36. The second sentence is to be amended to read:

(As noted in the Prohibition Section, the use of viruses classified [1] as Class 4 or 5 is prohibited.)

During the thirty-day comment period, no comments were received. The RAC discussed this issue at the June 5–6, 1980 meeting. It was suggested that the wording "beyond that which occurs by natural genetic exchange" in Section I–E should be added to Section III and Section IV–E–1–b–2–e following the clause "* * * which increases the virulence and host range of a plant pathogen." In addition, it was suggested that the language of Section IV–E–1–b–2–e shoud be clarified by addition of the phrase "assigning containment levels." A modified Section IV–E–1–b–2–e would read:

IV–E–1–b–2–e. Assigning containment levels for experiments with recombinant DNA from Class 3 organisms [1] and assigning containment levels for experiments which increase the host range and virulence of plant pathogens beyond that which occurs by natural genetic exchange.

With these modifications, the RAC accepted the proposals by a vote of fourteen in favor, none opposed, and three abstentions.

I accept these recommendations.

I–D. Proposal To Amend Section I–D–6 fo the Guidelines

In a letter of April 10, 1980, Dr. Irving Johnson of Lilly Research Laboratories proposed, among other changes, that Section I–D–6 of the Guidelines should be amended. The proposal would delete the second paragraph of Section I–D–6, which reads as follows:

We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale.

The proposal was published for thirty days of comment in the **Federal Register** of April 30, 1980 [45 FR 28908].

No comments concerning this proposal were received during the thirty-day comment period.

The RAC discussed this proposal at the June 5–6, 1980 meeting. It was pointed out that the probability of escape does not necessarily increase with increasing scale. The RAC noted that the paragraph constitutes prefatory language and that its deletion would not change the containment standards of the Guidelines.

By a vote of fourteen in favor, one opposed, and one abstention, the RAC recommended that the second paragraph of Section I–D–6 of the Guidelines be deleted.

I accept this recommendation.

I–E. Proposal To Amend Portions of Section III–A–2–a, Viruses of Eukaryotes

In the 1980 Guidelines a level of biological containment requiring the use of an "HV1 host and a vector certified for use in an HV2 system" was substituted for "an EK1 host and a vector certified for use in an EK2 system." This level of biological containment is abbreviated as "HV1CV" in Tabe III and Footnote 40 of the 1980 Guidelines. At the December 6–7, 1979 meeting of the RAC, a working group was appointed to review the appropriateness of this level of biological containment. The working group proposed that HV2 biological containment be required for these experiments. Accordingly, a proposal to amend portions of Sections III–A–2–a, Viruses of Eukaryotes, was published in the **Federal Register** of January 31, 1980 [45 FR 7182].

The RAC discussed this proposal at the March 6–7, 1980 meeting. During the discussion, the RAC agreed that the "CV" nomenclature, developed for EK host-vector systems, did not seem appropriate for HV systems in general. However, the RAC felt that requiring the use of HV2 systems instead of HV1CV imposed an unnecessarily stringent level of containment. The RAC voted to publish for comment in the **Federal Register** a proposal to sustitute HV1 for HV1CV in all places in the Guidelines.

The proposal as published in the **Federal Register** of April 30, 1980 reads as follows:

Substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system" in Section III–A–2–a–(1)–(a)–(3)–(b) and substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1," in Sections III–A–2–a–(1)–(a)–(2)–(c), III–A–2–a–(1)–(b)–(1)–(b), III–A–2–a–(1)–(b)–(2)–(b), III–A–2–a–(2)–(a)–(1)–(b), III–A–2–a–(2)–(a)–(2)–(b), and III–A–2–a–(2)–(c)–(2)–(b). In Table III, whenever any one of the following three terms appears, ("HV1CV [40]," "HV1CV[40] or P3 + HV1," or "HV1CV[40] or P3 + HV1[38]") it would be changed to "HV1".

No comments were received during the thirty-day comment period. The RAC at its June 5–6, 1980 meeting, once again examined this issue. A motion to table failed by a vote of two in favor, nine opposed, and three abstentions. By a vote of nine in favor, and five opposed, the RAC accepted these proposed changes as published in the **Federal Register.**

I accept this recommendation.

I–F. Proposal To Amend Section III–B–3 of the Guidelines

Dr. Allan Campbell of Stanford University proposed the the RAC consider amending Section III–B–3 of the Guidelines , which currently read as follows:

III–B–3. Non-HV1 systems. Containment levels for other classes of experiments involving non HV1 systems may be approved by the Director, NIH. [See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b)].

In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 Containment. (2A)

Dr. Campbell stated that the P3 containment level was assigned to this type of experiment because of apprehensions that the category might contain some experiments for which that level was appropriate, rather than that all experiments in this category were perceived as requiring P3 containment. He proposed amending this section to "assert RAC's intention to review individual proposals in an open-minded manner with respect to realistic evaluation of possible hazard, and to discourage the inference that the P3 level, as it applies to a specific experiment, has been set on the basis of information about the organisms involved."

Dr. Campbell's proposed changes in the Guidelines were published in the **Federal Register** of April 30, 1980 [45 FR 28906] for thirty days of public comment, as follows:

A. Section III–B–3, paragraph 2, would be amended to read:

In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment [2A]. Lower levels of physical containment may be assigned by NIH for specific donor-receipient combinations (See Section IV–E–1–b–2–(f).

B. A new Section IV–E–1–b–2–(f) would be added to the Guidelines as follows:

IV–E–1–b–2–(f). Assigning containment levels for experiments in which both donor and recipient are non-pathogenic prokaryotes (See Section III–B–3).

No comments were received during the thirty-day comment period. The RAC discussed this issue at the June 5–6, 1980 meeting. During the discussion, Dr. Campbell said that the term "non-pathogenic" refers to CDC Class 1 etiological agents. It was noted that the proposal simplifies procedures by removing the requirement that proposals to lower physical containment levels for

specific donor-recipient combinations falling under Section III–B–3 be published in the **Federal Register** for public comment, prior to review by the RAC.

The RAC recommended acceptance of this proposal by a vote of thirteen in favor, none opposed, and four abstentions.

I accept this recommendation.

I–G. Proposal To Amend Section III–C–4 of the Guidelines

Dr. Milton Zaitlin proposed at the March 6–7, 1980 RAC meeting that Section III–C–4 of the Guidelines be amended to redefine a P1 greenhouse or growth chamber when certain infectious agents other than viruses are used as vectors.

Dr. Zaitlin noted that the description of a P1 greenhouse or growth chamber in Section III–C–3, Plant Viral Host-Vector Systems, recommends positive air pressure be maintained in order to exclude insects which might transmit viruses. However, in the conditions considered in Section III–C–4, i.e., Plant Host-Vector Systems other than Viruses, the propagules themselves (spores, mycelia, etc.) should be contained, and negative air pressure would be more appropriate.

He proposed that the following language be added at the end of Section III–C–4:

and (iii) negative air pressure should be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules.

This proposal was published in the **Federal Register** of April 30, 1980 [45 FR 28906]. No comments were received during the thirty-day comment period.

The RAC considered this proposal at the June 5–6, 1980 meeting. The proposal was approved by a vote of fourteen in favor, and none opposed.

I accept this recommendation.

I–H. Request To Delete Language Requiring the Use of Cauliflower Mosaic Virus (CaMV) Mutants Lacking the Aphid Transmission Factor From Section III–C–3. Request for Permission To Transfer DNA Fragments From Aphid Transmissible to Aphid Nontransmissible CaMV

In a letter of January 17, 1980, Dr. Robert J. Shepherd of the University of California at Davis requested that paragraph four of Section III–C–3 of the Guidelines be deleted. This paragraph reads as follows:

The CaMV strain used as a cloning vector shall be a mutant that lacks the aphid transmission factor.

Dr. Shepherd suggested that it is inconsistent to restrict studies with cauliflower mosaic virus to non-insect transmissible strains when no such restriction is imposed on studies with bean golden mosaic virus and related viruses, the only other known group of DNA plant viruses. Dr. Shepherd noted that, under the P1 containment conditions specified in the Guidelines, cauliflower mosaic virus is easily confined to a growth chamber or greenhouse. He added that CaMV has a very restricted host range and is neither seed nor pollen transmitted.

Dr. Shepherd also requested permission to introduce DNA segments from aphid transmissible strains to non-aphid transmissible strains of CaMV in order to study the factors determining aphid transmissibility.

A notice outlining Dr. Shepherd's request appeared in the **Federal Register** of April 30, 1980 [45 FR 28906] for thirty days of public comment.

No comments were received during the thirty-day comment period.

The RAC discussed Dr. Shepherd's proposal at the June 5–6, 1980 meeting. By a vote of ten in favor, none opposed, and four abstentions, the RAC recommended that paragraph four of Section III–C–3 be deleted from the Guidelines. By a vote of eleven in favor, none opposed, and three abstentions, the RAC recommended approval of Dr. Shepherd's request for permission to introduce DNA segments from aphid transmissible strains to the genome of non-aphid transmissible strains of CaMV.

I accept these recommendations.

I–I. Status of Variola and Whitepox Viruses

At the March 6–7, 1980 meeting of the RAC, the suggestion was made that any mention in the Guidelines of the variola viruses be marked with a symbol so that reference could be made to World Health Organization policy regarding worldwide efforts at eradicating these viruses.

Accordingly, it was proposed that Appendix B, *Classification of Microorganisms on the Basis of Hazard,* be amended so that each mention of Alastrim, Smallpox and Whitepox will be marked with a symbol. With the concurrence of the Center for Disease Control, the following footnote, to be added to Appendix B, appeared in the **Federal Register** of April 30, 1980 [45 FR 28908] for thirty days of comment:

All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta).

During the thirty-day comment period, no comments were received. The RAC at its June 5–6, 1980 meeting recommended that the marking of any mention of Alastrim, Smallpox and Whitepox in Appendix B was appropriate, and approved the proposed language of the footnote by a vote of seventeen in favor, and none opposed.

I accept this recommendation.

I–J. Transfer of DNA Segments Cloned in Saccharomyces Cerevisiae Host-Vector Systems to Higher Plants

Dr. Jane Setlow of Brookhaven National Laboratory suggested that the Guidelines include a general provision for cloning plant DNA in *Saccharomyces cerevisiae* and subsequently returning the cloned DNA to the plant. The following proposed amended language of the first sentence of Section III–C–7–b appeared in the **Federal Register** of April 30, 1980 [45 FR 28908]:

III–C–7–b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I–D] which has been cloned and propagated in *E. coli* or *S. cerevisiae* under appropriate containment conditions, may be transferred with the *E. coli* or *S. cerevisiae* vector used for cloning to any higher plant organisms (Angiosperms and Gymnosperms) and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A].

During the thirty-day comment period, no comments were received.

The RAC discussed this proposal at the June 5–6, 1980 meeting. The proposal was accepted by a vote of twelve in favor, none opposed, and six abstentions.

I accept this recommendation.

The language of the **Federal Register** announcement of April 30, 1980 [45 FR 28908] differed from the language of the revised Guidelines promulgated on January 29, 1980 [45 FR 6738]; the latter specify "P1 conditions," while the former said "appropriate containment conditions." The phrase "appropriate containment" had been published in the April 30, 1980 **Federal Register** announcement as the proposal to include laboratory strain *S. cerevisiae* host-vector systems under Section III–O had not yet been considered by the RAC and the NIH. The latter proposal is now being accepted, and new language for Section III–O is being promulgated in this document. As laboratory strain *S. cerevisiae* host-vector systems will henceforth be included under Section III–O, the language stipulating P1 containment conditions, being more

precise, will be used in Section III–C–7–b.

I–K. Request for Consideration of Appropriate Containment Conditions

Dr. Paul S. Sypherd of the University of California, Irvine, requested in a letter of May 13, 1980 that the RAC evaluate containment conditions appropriate to the return of *Mucor racemosus* DNA cloned in *Saccharomyces cerevisiae* host-vector systems to *Mucor racemosus*. In Addition, Dr. Sypherd requested permission to transform *Mucor racemosus* with *S. cerevisiae* vectors with or without cloned *S. cerevisiae* sequences. Dr. Sypherd noted that *Mucor* species in general, and *M. racemosus* in particular, are ubiquitous. *M. racemosus* is globally distributed and is found in air, in soil, on fruits, and in animal dung. He added that *M. racemosus* is nonpathogenic and produces no known toxins. *M. racemosus* is not known to mate with other species of *Mucor*, and mating within the species occurs at a very low frequency under precisely defined conditions. A Federal Register announcement of Dr. Sypherd's request appeared on April 30, 1980 [45 FR 28908].

During the thirty-day comment period, no comments were received.

During the discussion of this issue at the June 5–6, 1980 meeting, it was noted that certain species of *Mucor* can cause mycoses in medically compromised humans. It was thus suggested that the principal investigator be alerted to the pathogenic potential of certain *Mucor* species.

By a vote of fourteen in favor, none opposed, and four abstentions, the RAC recommended acceptance of Dr. Sypherd's proposal at the P2 containment level.

I accept this recommendation.

I–L. Cloning of Foot and Mouth Disease Virus—Stage II

The RAC at its June 5–6, 1980 meeting considered the request from scientists at the Plum Island Animal Disease Center to remove clones containing sequences equivalent to a portion of the genome of the Foot-and-Mouth Disease (FMD) Virus from Plum Island for further development at Genentech, Inc., of South San Francisco, California. This is Stage II of a cooperative recombinant DNA project by scientists at both institutions to clone segments of the Foot-and-Mouth Disease Virus for the purpose of developing a vaccine derived from the VP_3 protein encoded by the virus. Stage I of the project, involving the construction and analysis of a gene bank of plasmids containing FMD sequences, had been completed at Plum Island. In accord with the recommendations of the RAC at its December 6–7, 1979 meeting, and the approval of the Director, NIH, the investigators submitted data to demonstrate that the clones to be removed from Plum Island to Genentech, Inc. are noninfective, and contain sequences in aggregate less than the full genome of the FMD virus.

Announcement that the RAC would review the request to remove clones from Plum Island and would recommend containment levels for the work at Genentech, Inc., appeared in the Federal Register of April 30, 1980. No comments were received during the thirty-day comment period.

Information on the characterization of the inserted DNA sequences was provided to the RAC to support the RAC's stipulation that "clones to be approved for removal from Plum Island shall not contain among them collectively or individually, the full genome of the FMD virus." (A full discussion of the RAC's previous recommendations appears in 45 FR 3552.) The RAC further considered the evidence presented which demonstrated that the clones lack infectivity. The hybridization and restriction analysis data for characterization were found satisfactory.

On the basis that the 17 clones described in the submission were well characterized, lacked infectivity, and represent, in aggregate, only 75% of the FMD viral genome, a motion was passed to recommend approval for removal of these clones from Plum Island by a vote of twelve in favor, two opposed, and four abstentions.

A motion to permit work with the clones at Genentech, Inc. under P1 + EK1 conditions passed by a vote of eleven in favor, one opposed, and five abstentions.

I accept these recommendations.

I–M. Lowering of Containment Levels for Characterized Clones or Purified DNAs by Institutional Biosafety Committees

The RAC at its March 6–7, 1980 meeting voted nine in favor, one opposed, with three abstentions, that there be published in the Federal Register for comment, and brought back to the RAC at its June 5–6, 1980 meeting, proposed revisions of the Guidelines to permit a single-step reduction in physical or biological containment for characterized clones or purified DNA, by the local Institutional Biosafety Committee (IBC), without a requirement for NIH approval. Accordingly, the following proposed changes in the Guidelines were published in the Federal Register of April 30, 1980:

A. *Section III–A–3–a is to be amended as follows:*

III–A–3–a. *Purified DNA Other than Plasmids, Bacteriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNAs that have been purified [41] and in which the absence of harmful sequences has been established [3] can be carried out under lower containment conditions than used for the corresponding shotgun experiment [42]. The containment may decrease one step in physical containment [P4→P3; P3→P2; P2→P1] while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment [HV3→HV2; HV2→HV1] while maintaining the specified physical containment. The institutional biosafety committee (IBC) must review such a reduction and the approval of the IBC must be secured before such a reduction may be put into effect. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1 + HV1, which requires prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–(3)–(e).)

B. *Section III–A–3–b is to be amended as follows:*

III–A–3–b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (3) experiments involving this recombinant DNA may be carried out under lower containment conditions.

Institutional biosafety committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III—A—3—b to levels below P1+HV1, or reduction of containment levels by more than one step, which also require prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–3–(e).)

C. *Section IV–D–1–c–(3) is to be amended as follows:*

IV–D–1–c–(3). Certain reductions of containment levels for characterized or purified DNA preparations or clones (see Section III–A–3).

D. *The Note in Section IV–D–3–b is to be amended as follows: (Additional language in Italics).*

Note.—Some examples of work that might ordinarily proceed without prior funding-agency approval are the initiation of a project at the P1 or P2 level (other than the first project at the institution). Other example are significant changes in hosts or vectors, in the donor species or the nature of the DNA segment selected, or in the physical location of the experiments. *Still others are single-step reductions in containment level for (i) experiments with DNA recombinants from cellular DNAs that have been pruified and are judged to be free of harmful sequences (See Section III–A–3–a) and for (ii) clones that have been characterized and judged to*

be free of harmful sequences (see Section III–A–3–b). It should be clear, however, that the funding agency must be notified of IBC approvals even when prior agency approval is not required. See the Administrative Practices Supplement for further discussion.

No comments were received during the thirty-day comment period. These proposed changes of the Guidelines were considered by the RAC at its June 5–6, 1980 meeting. The RAC recommended the changes by a vote of fifteen in favor, none opposed, and no abstentions.

I accept the proposed changes.

I-N. Request for Permission To Transform Saccharomycopsis Lipolytica With E. Coli/S. Cerevisiae Hybrid Plasmids

In response to a request to approve the transformation of the yeast *Saccharomycopsis lipolytica* with defined *E. coli/Saccharomyces cerevisiae* hybrid plasmids the following notice appeared in the April 30, 1980 **Federal Register:**

"Dr. David M. Ogrydziak of the University of California, Davis, requests permission to attempt to transform the yeast *Sacchromycopsis lipolytica* with defined *Escherichia coli/Saccharomyces cerevisiae* hybrid plasmids and to use the hybrid plasmids for shotgunning *S. lipolytica* DNA in *E. coli* and returning the DNA to *S. lipolytica.*

During the thirty-day comment period there were no comments. The RAC discussed the proposal during its June 5–6, 1980 meeting. It was noted that *S. lipolytica* is not a pathogen, and does not colonize animals. A Motion to approve these experiments at the P1 level of containment was passed by a vote of fifteen in favor, none opposed, and one abstention.

I accept this recommendation.

I–O Request for Use of Conjugative Plasmids and Transducing Phages in an Escherichia Coli K–12 Host.

The following notice was published in the **Federal Register** of April 30, 1980:

Dr. A. I. Bukhari of the Cold Spring Harbor Laboratory has requested permission from the RAC to employ conjugative plasmids or transducing phages in recombinant DNA experiments involving *Escherichia coli* DNA when a small fragment of heterologous DNA is used as a linker. In the specific request, Dr. Bukhari requests approval to use a fragment of Adenovirus 2 DNA as linker DNA.

No comments were received during the thirty-day comment period.

In the RAC discussion of the request at its June 5–6, 1980 meeting, it was noted that the small Adenovirus DNA linker segments of 1 kb size were not derived from the transforming region and could, therefore, be treated as inert DNA. The motion recommending approval of this request passed by a vote of fourteen in favor, none opposed, and one abstention.

I accept this recommendation.

II. Summary of Major Actions Under Guidelines

II–A. Amendment of Section I–D–1

Section I–D–1 is amended to read:

I–D–1. Formation of recombinant DNAs derived from pathogenic organisms classified [1] as Class 4 or 5 or from cells known (2A) to be infected with such agents, regardless of the host-vector system used.

II–B. Deletion of Section I–D–3

Section I–D–3 is deleted. Substitute for the current wording, the word "deleted."

II–C. Amendment of Section I–D–6 of the Guidelines

Delete the second paragraph of Section I–D–6, which reads as follows:

We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale.

II–D. Amendment of Section I–E

Section I–E is amended to read:

I–E. Exemptions. It must be emphasized that the following exemptions [4] are not meant to apply to experiments described in Sections I–D–1 to I–D–5 as being prohibited. In addition, any recombinant DNA molecules involving DNA from Class 3 organisms [1] or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange, are not exempt unless specifically so designated by NIH under Section I–E–5.

II–E. Amendment of Section III

After the last sentence of Section III, "The use of higher levels of biological containment * * * for the purposes of the experiment," the following new paragraph is to be added:

Experiments involving recombinant DNA from Class 3 organisms [1] or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K–12 EK1 hosts (see Section III–O). Containment levels for all other experiments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange will be determined by NIH. (See Section IV–E–1–b–2–e).

II–F. Amendment of Section III–O of the Guidelines

Section III–O of the Guidelines is amended to read as follows: [This amended language combines (1) the language of the proposal to include *Saccharomyces cerevisiae* host-vector systems under Section III–O, (2) the language of the proposal to include Ff phages under Section III–O, and (3) a paragraph pertaining to CDC Class 3 agents and certain plant pathogens.]

III–O. *Classification of Experiments Using E. coli K–12 and Saccharomyces cerevisiae Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K–12 host-vector systems; others employ the *S. cerevisiae* host-vector systems. These are the systems for which we have the most experience and knowledge.

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are prohibited (see Section I–D).

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are exempt from the Guidelines (see Section I–E).

Experiments using *E. coli* K–12 host-vector systems and DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment or at a lower level as specified by NIH (See Section IV–3–1–b–2–e).

Other experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, an HV1 hostvector system [i.e., for experiments using *E. coli* K–12 (a) the *E. coli* host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid of Ff bacteriophages or non-conjugative plasmids [49] shall be used as vestors. For experiments in *S. cerevisiae,* laboratory strains shall be used]. For these experiments no Memorandum of Understanding and Agreement (MUA) as described in Section IV–D–1–c need be submitted, nor is any registration with NIH necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, and (c) the hosts and vectors to be used. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals but such review is not required prior to initiation of experiments. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express as a protein product the information carried in any gene derived from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.

Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).

For those not on Appendix A list but which exchange genetic information [35] with *E.*

coli, experiments may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a nonconjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or intergrated, or generalized transducing phages."

II–G Amendment of Section III–A–2–a, Virsuses of Eukaryotes

Substitute the term "an HV1 host-vector" or "an HV1 host and a vector certified for use in an HV2 system" in Section III–A–2–a–(1)–(a)–(3)–(b) and substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1," in Sections III–A–2–a–(1)–(a)–(2)–(c), III–A–2–a–(1)–(b)–(1)–(b), III–A–2–a–(1)–(b)–(2)–(b), III–A–2–a–(2)–(a)–(1)–(b), III–A–2–a–(2)–(a)–(2)–(b), and III–A–2–a–(2)–(c)–(2)–(b). In Table III, whenever any one of the following three terms appears, ("HV1CV[40]"; "HV1CV[40] or P3 + HV1;" or "HV1CV[40] or P3 + HV1[38]") substitute "HV1."

II–H. Section III–A–3–a Is Amended as Follows

III–A–3–a. *Purified DNA Other Than Plasmids, Bacteriophages, and Other Viruses.* The information of DNA recombinants from cellular DNAs that have been purified [41] and in which the absence of harmful sequences has been established [3] can be carried out under lower containment conditions than used for the corresponding shotgun experiment [42]. The containment may be decreased one step in physical containment [P4→P3; P3→P2; P2 P1] while maintaining the biological containment specified for the shotgun experiement, or one step in biological containment [HV3→HV2; HV2→HV1] while maintaining the specified physical containment. The institutional biosafety committee (IBC) must review such a reduction and the approval of the IBC must be secured before such a reduction may be put into effect. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1 + HV1, which requires prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–(3)(e).)

II–I. Section III–A–3–b Is Amended as Follows

III–A–3–b. *Characaterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (3) experiments involving this recombinant DNA may be carried out under lower containment conditions.

Institutional biosafety committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone dervied from a shotgun experiment and its probable freedom from harmful genes. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III-A-3-b to levels below P1 + HV1, or reduction of containment levels by more than one step, which also requires prior NIH approval. (See Sections IV-D-1-c and IV-E-1-b-3-(e).)

II-J Amendment of Section III-B-3

Section III-B-3, paragraph 2 is amended to read:

In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment [2A]. Lower levels of physical containment may be assigned by NIH for specific donor-recipient combinations (See Section IV-E-1-b-2-(f)).

II-K. Amendment of Section III-C-3 of the Guidelines

Delete paragraph four of Section III-C-3, which reads as follows:

The CaMV strain used as a cloning vector shall be a mutant that lacks the aphid trasmission factor.

II-L. Amendment of Section III-C-4 of the Guidelines

Paragraph two of Section III-C-4 is amended by changing the end of the paragraph from "* * * work." to "* * * work, and (iii) negative air pressure should be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules."

II-M. Amendment of Section III-C-7-b of the Guidelines

Section III-C-7-b is amended to read as follows:

III-C-7-b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I-D] which has been cloned and propagated in *E. coli* or *S. cerevisiae under P1 conditions, may be transferred with the E. coli* or *S. cerevisiae* vector used for cloning to any high plant organisms (angiosperms and Gymnosperms) and propagated under condition of physical containment comparable to P1 and appropriate to the organism under study (2A). Intact plants or propagative plant parts may be grown under P1 conditions described under Section III-C-3. Containment must be modified to ensure that the spread of pollen, seed or other propagules is prevented. This can be accomplished by conversion to negative pressure in the growth cabinet or greenhouse or by physical entrapment by "bagging" of reproductive structures. Transfers to any other plant organisms will be considered on a case-by-case basis (45).

II-N. Section IV-D-1-c-(3) Is Amended as Follows

IV-D-1-c-(3). Certain reductions of containment levels for characterized or purified DNA preparations or clones (see Section III-A-3).

II-O. Amendment of Section IV-D-3-b

The note in Section IV-D-3-b is amended as follows:

Note.—Some examples of work that might ordinarily proceed without prior funding-agency approval are the initiation of a project at the P1 or P2 level (other than the first project at the institution). Other examples are significant changes in hosts or vectors, in the donor species or the nature of the DNA segment selected, or in the physical location of the experiments. Still others are single-step reductions in containment level for (i) experiments with DNA recombinants from cellular DNAs that have been purified and are judged to be free of harmful sequences (See Section III-A-3-a) and for (ii) clones that have been characterized and judged to be free of harmful sequences (see Section III-A-3-b). It should be clear, however, that the funding agency must be notified of IBC approvals even when prior agency approval is not required. See the Administrative Practices Supplement for further discussion.

II–P. Addition of Section IV–E–1–b–2–(e)

A new Section IV–E–1–b–2–(e) is added as follows:

IV–E–1–b–2–(e). Assigning containment levels for experiments with recombinant DNA from Class 3 organisms [1] and assigning containment levels for experiments which increase the host-range and virulence of plant pathogens beyond that which occurs by natural genetic exchange.

II–Q. Addition of Section IV–E–1–b–2–(f)

A new Section IV–E–1–b–2–(f) is added a follows:

IV–E–1–b–2–(f). Assigning containment levels for experiments in which both donor and recipient are non-pathogenic prokaryotes (See Section III–B–3).

II–R. Amendment of Footnote 2, Section V

Section V, footnote 2, is amended to read:

2. For experiments using Vesicular Stomatitis virus (VSV), contact the NIH Office of Recombinant DNA Activities.

II–S. Amendment of Footnote 36, Section V

The second sentence of footnote 36, Section V is amended to read:

(As noted in the Prohbition Section, the use of viruses classified [1] as Class 4 or 5 is prohibited.)

II–T. Status of Variola and Whitepox Viruses

A new footnote is added to Appendix B of the Guidelines as follows:

* * * All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization (WHO) Collaborating Center

for Smallpox Research, Center for Disease Control, in Atlanta).

Each mention of Alastrim, Smallpox and Whitepox in Appendix B should be followed by the symbol * * *.

II–U. Amendment of Appendix D

Appendix B is amended to read:

As noted above at the beginning of Section III–A, certain HV1 and HV2 host-vector systems are assigned containment levels as specified in the subsections of Section III–A. Those so classified as of publication of these Revised Guidelines are listed below.

HV1*—The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:

(1) inl (inositolless) strains 37102, 37401, 46316, 64001 and 89601.

(2) csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

(3) eas strain UCLA191 (an "easily wettable" mutant).

HV1—Asporogenic mutant derivatives *B. subtilis*. The *B. subtilis* HV1 derivatives must not revert to sporeformers with a frequency greater than 10^{-7}; data confirming this requirement must be presented to NIH for certification. The following plasmids are accepted as the vector components of certified *B. subtilis* HV1 systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223. *B. subtilis* strain RUB 331 has been certified as the host component of HV1 systems based on these plasmids.

II–V. Amendments of Appendix E

The following sections are added to Appendix E:

- Certain specified clones derived from segments of the Foot-and-Mouth Disease Virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Francisco, California. Further development of the clones at Genentech has been approved under P1 + EK1 conditions.
- *Sacchromycopsis lipolytica* may be used as a host for transformation with defined *Escherichia coli/Saccharomyces cerevisiae* hybrid plasmids and the hybrid plasmids may be used for cloning *S. lipolytica* DNA in *E. coli* and returning the cloned DNA to *S. lipolytica*.
- Conjugative plasmids or transducing phages may be employed in recombinant DNA experiments when employing *E. coli* as host when a small defined segment of Adenovirus 2 DNA is employed as linker DNA.
- Permission is granted to introduce DNA segments from aphid transmissible strains into non-aphid transmissible strains of Cauliflower mosaic virus in order to study the factors determining aphid transmissibility.
- Permission is granted to return *Mucor racemosus* DNA which has been cloned in *Saccharomyces cerevisiae* host-vector systems to *Mucor racemosus*. In addition, permission is granted to transform *Mucor racemosus* with *S. cerevisiae* vectors with or without cloned *S. cerevisiae* sequences. These manipulations may be performed under P2 conditions.
- Many experiments utilizing *E. coli* or *S. cerevisiae* host-vector systems are now covered by Section III–O of the Guidelines. This section states, in part, that "* * * experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, an HV1 host/vector system * * *"

While the Guidelines no longer specifiy the use of EK2 or *S. cerevisiae* HV2 systems, investigators may wish to employ these systems in specific instances. The currently certified EK2 and HV2 systems are:

HV2—The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32 and YIp33.

EK2 Plasmid Systems. The *E. coli* K–12 strain chi–1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR327. The following *E. coli/S. cerevisiae* hybrid plasmids are certified as EK2 vectors when used in *E. coli* chi–1776 or in the sterile yeast strains, SHY1, SHY2, SHY3 and SHY4: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, YIp33.

EK2 Bacteriophage Systems. The following are certified EK2 Systems based on bacteriophage lambda:

Vector	*Host*
λ gt*WES*. λ B'	DP50*sup*F
λ gt*WES*. λ B*	DP50*sup*F
λ gtr*ZIvin*. λ B'	*E. coli* K–12
gtALO. λ B	DP50*sup*F
Charon 3A	DP50 or DP50*sup*F
Charon 4A	DP50 or DP50*sup*F
Charon 16A	DP50 or DP50*sup*F
Charon 21A	DP50*sup*F
Charon 23A	DP50 or DP50*sup*F
Charon 24A	DP50 or DP50*sup*F

*These follow the assigned containment levels as specified in the subsections of Section III–A with one exception. This exception is that experiments involving complete genomes of eukaryotic viruses will require P3 + HV1 or P2 + HV2 rather than the levels given in the subsections of Section III–A.

Additional Announcements of the Director, NIH

In addition, two further changes are being promulgated in order to clarify the intent of the language in two sections of the Guidelines.

I. Addition of Subheading to Section I–D

A new subheading, "I–D (1–6)," is to be added to the penultimate paragraph in Section I–D to emphasize that this paragraph applies to I–D–1 through I–D–6. The amended paragraph reads as follows:

I–D (1–6). Experiments in Categories I–D–1 to I–D–6 may be excepted (4) from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provided that these experiments are expressly approved by the Director, NIH, with advice of the Recombinant DNA Advisory Committee after appropriate notice and opportunity for public comment (See Section IV–E–1–b–(1)–(e).)

II. Amendment of Appendix E, Item 6

Item 6 in Appendix E is amended to read as follows:

Streptomyces coelicolor can be used as a host for the cloning of DNA derived from *B. subtilis*, *E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis* under P2 conditions, using as a vector any plasmid indigenous to Streptomyces coelicolor or able to replicate in that host by natural biological mechanisms.

Dated: July 18, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8(b) (4) and (5) of that Circular.

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